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(71) Applicant: ABBOTT LABORATORIES [US/US]; Chad 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).

(72) Inventors: LI, Luk, C.; 5 Birmingham Place, Vernon Hills, IL 60061 (US). PEC, Edward, A.; 3544 S. Vernon, Brookfield, IL 60513 (US). PARASRAMPURIA, Jagdish; 1539 Parrott Drive, San Mateo, CA 94402 (US).

(74) Agents: WEINSTOCK, Steven, F. et al.; Abbott Laboratories, CHAD 0377/AP6D-2, 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).

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(54) Title: STABLE AQUEOUS FORMULATIONS OF 1α,25-DIHYDROXYCHOLECALCIFEROL FOR PARENTERAL ADMINISTRATION

(57) Abstract

1α,25-dihydroxycholecalciferol is a naturally occurring form of Vitamin D_3. Vitamin D_3 is converted to 1α, 25-dihydroxycholecalciferol in the liver and kidney before it acts to stimulate intestinal calcium and phosphorous absorption. Parenteral solutions of 1α, 25-dihydroxycholecalciferol are suitable for replacement therapy.

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STABLE AQUEOUS FORMULATIONS OF 1α, 25-DIHYDROXYCHOLECALCIFEROL FOR PARENTERAL ADMINISTRATION

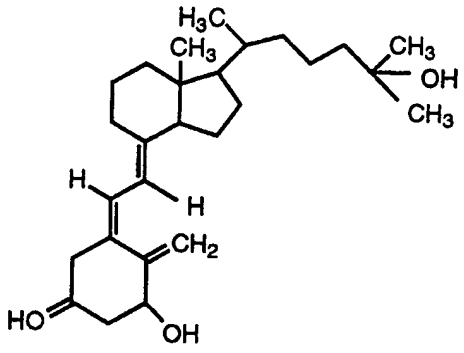
BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to aqueous formulations of 1α,25-dihydroxycholecalciferol in parenteral solutions.

2. Discussion of the Prior Art

1α,25- Dihydroxycholecalciferol is represented by the formula:



The compound has a molecular weight of 416.647, a molecular formula of $C_{27}H_{44}O_3$, is soluble in organic solvents and is practically insoluble in water. The generic name of 1α,25-dihydroxycholecalciferol is calcitriol.

1α,25-dihydroxycholecalciferol stimulates intestinal calcium and phosphorus absorption and, with parathyroid hormone, stimulates bone calcium resorption or mobilization. 1α,25-dihydroxycholecalciferol meets the criteria of a classic steroid hormone in that it is synthesized in one organ under closely regulated conditions, and is then transported by the circulation to another organ where it exerts its biological effects.

The discovery that the kidney is the exclusive site for the production of 1α,25-dihydroxycholecalciferol from 20-hydroxycholecalciferol provided an explanation for the vitamin D-resistant state observed in patients with chronic uremia. Blood levels of 1α,25-dihydroxycholecalciferol in patients with chronic renal failure are low or undetectable. The successful chemical synthesis of 1α,25-dihydroxycholecalciferol made this metabolite available for replacement therapy, and its clinical use for the treatment of hypocalcemia in patients on renal dialysis has recently been approved.

The effectiveness of this drug in several other clinical conditions has been reported in the scientific literature. These uses of 1α,25-dihydroxycholecalciferol include the treatment of renal osteodystrophy, hypoparathyroidism, osteomalacia, osteoporosis, hepatic osteodystrophy, vitamin D-resistant rickets, vitamin D-dependent rickets, childhood renal failure and neonatal hypocalcemia.

Neonatal hypocalcemia is the most prevalent hypocalcemic state encountered in pediatrics and can be divided into two main groups: (1) "early" neonatal hypocalcemia beginning in the first 48 hours of life and (2) "late" neonatal hypocalcemia beginning at the end of the first week of life.

Nearly all infants experience a normal fall in serum calcium during the first few days of life. Early neonatal hypocalcemia appears to be an accentuation of this normal fall in serum calcium, and is often defined as a serum calcium level of ≤7 milligrams/deciliter (mg/dl) or ≤8 mg/dl for full term infant or a serum ionized calcium level of from about 3 to 3.5 mg/dl. Minimal serum calcium levels are reached at 24-48 hours of age with a gradual return to normal in the next few days. Early neonatal hypocalcemia is frequently accompanied by hyperphosphatemia. In unusual circumstances, early neonatal hypocalcemia may persist for a week or more, and this condition has been called transient congenital idiopathic hypoparathyroidism. Approximately one-third of premature

infants (≤7 weeks gestation), one-third of infants with birth asphyxia (1 minute apgar score of ≤6) and one-half of infants of insulin-dependent diabetic mothers have early neonatal hypocalcemia.

Low serum ionized calcium level is associated with serious signs including seizures, apnea, vomiting, neuromuscular irritability, gastric atony, cyanosis and lethargy. Hypocalcemia can also occur without signs of neuromuscular hyperirritability. Correlation of clinical signs with serum calcium levels has been difficult because of the many clinical variables coexistent with hypocalcemia in these high risk infants.

Late neonatal hypocalcemia usually occurs in full term or premature infants who have been started on feedings and who show signs or symptoms of hypocalcemia only after several days or weeks of feedings. The hypocalcemia appears to be precipitated by the high phosphate load of most feedings other than human milk. The high phosphate serum concentration in serum of infants in the first few weeks is associated with low parathyroid hormone levels and with a low glomerular filtration rate.

Chronic renal dialysis patients can also undergo hypocalcemia treatment. Most patients undergoing hemodialysis respond to treatments for hypocalcemia three times per week,

Calcijex® (Abbott Laboratories, Abbott Park, IL. 60034), a formulation containing 1α, 25 - dihydroxycholecalciferol in an aqueous formulation, is one example suitable for parenteral administration for both neonatal hypocalcemia and hemodialysis. For example, hemodialysis patients can be treated with initial doses of 1α, 25 - dihydroxycholecalciferol approximately every other day. Serum calcium and phosphorus levels are obtained regularly to monitor the patient until normal calcium levels are observed. Generally, patient dosage of 1α, 25 - dihydroxycholecalciferol is limited to a total of 9 micrograms per week.

Chelating agents, including but not limited to citric acid, tartaric acid, amino acids, thioglycolic acid, and edetate disodium (EDTA), and buffers, including but not limited to acetate, citrate, glutamate, and phosphate buffers, are often used to stabilize formulations containing drugs such as 1α, 25 - dihydroxycholecalciferol. However, these additives are found to impart aluminum levels in products to over 3.5 parts per million at the expiration date of the product. The source of aluminum may be partially due to the buffer itself. The use of buffers and chelating agents may also increase aluminum levels by extracting aluminum from the glass ampoule which contains the formulation.

It would be particularly advantageous to minimize aluminum levels in formulations to minimize the risk of aluminum accumulation in dialysis patients which may lead to osteomalacia. Potential adverse effects of EDTA may also include nephrotoxicity and renal tubular necrosis. Furthermore, EDTA is a chelating agent which is not an acceptable injectable excipient in some international markets, such as Japan.

The formulations of the present invention are essentially free of EDTA and buffers which results in a reduction of aluminum levels in the formulations. In addition, some of the formulations of the present invention may be terminally sterilized by autoclaving, which imparts a 10^3 fold increase in sterility assurance level (SAL) of the final product over aseptic filling techniques. Parenteral products manufactured having a high SAL reduces patient exposure to potential infections.

SUMMARY OF THE INVENTION

The present invention provides pharmaceutical formulations having a pH of from about 5.8 to about 7.8 comprising a therapeutically effective amount of 1α, 25 dihydroxycholecalciferol, a solubilizing agent, an antioxidant, and are essentially free of a buffer and a chelating agent

wherein at least a two fold decrease in aluminum content results. The present invention also provides for sterile, aqueous solutions having a pH of from about 5.8 to about 7.8 comprising 1α, 25-dihydroxycholecalciferol, a solubilizing agent and an antioxidant wherein the aqueous solution has at least a two fold decrease in aluminum content as compared to solutions also containing a buffer and/or a chelating agent. Processes for preparing such sterile, aqueous solutions are also disclosed.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a dilute, stable, sterile, aqueous formulation of 1α, 25-dihydroxycholecalciferol suitable for parenteral administration. In the case of parenteral administration, the intramuscular route is preferred.

Generally, the dilute, stable, sterile, aqueous formulations comprise 1α, 25-dihydroxycholecalciferol, a solubilizing agent, and an antioxidant and are essentially free of a buffer and a chelating agent thereby providing at least a two fold decrease in aluminum content. Preferably, the formulations of the present invention are completely free of buffers and chelating agents.

The pH of the formulation may be controlled by the addition of hydrochloric acid and/or sodium hydroxide. Typically, the pH of the final product can range from about 5.8 to about 7.8.

The solubilizing agents which may be used in the formulations of the present invention comprise dimethylacetamide, polyethylene glycol 400 (PEG 400), polyethylene glycol 200 (PEG 200), ethanol, isopropanol, 1,3-butanediol, propylene glycol, dimethylsulfoxide, glycerin, water, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80. It is to be understood by those skilled in the arts that the solubilizing agents used in the formulations of the present invention must be of a pharmaceutically acceptable grade.

Preferably, the solubilizing agents used in the formulations of the present invention are nonionic surfactants which generally comprise the polyoxalkylene compounds, e.g., the mono-fatty acid esters of polyethylene glycol, the partial esters of fatty acids and polyhydric alcohols, or the anhydrides of such alcohols, etherified with polyalkylene oxides. In particular compounds such as polyoxyethylenesorbitan monolaureate (Tween® 20) and analogous compounds work well due to their solubilizing effectiveness and low toxicity. Other polyoxyethylene ester solubilizing agents which can be used include those listed in, but not intended to be limited to, the 1994 Sigma® Chemical Company catalog on pages 850-852. Those skilled in the art will recognize that a variety of pharmaceutically acceptable nonionic surfactants will work with the formulations of the present invention.

The amount of solubilizing agent used with formulations containing 1α, 25-dihydroxycholecalciferol will depend on the particular solubilizing agent used in the formulation. Generally, the solubilizing agent is used to help solubilize the 1α, 25-dihydroxycholecalciferol but is not toxic at the concentrations used. The solubilizing agents are used in making aqueous formulations containing the potent and toxic compound 1α, 25-dihydroxycholecalciferol. In the formulations of the present invention, a concentrate of 1α, 25-dihydroxycholecalciferol and solubilizing agent was made to provide a form of 1α, 25-dihydroxycholecalciferol which was easier to formulate.

Antioxidants are used to help prevent oxidation of the drug in the product. In the formulations of the present invention, the antioxidant is quickly oxidized thereby minimizing oxidation of 1α, 25-dihydroxycholecalciferol. Antioxidants and some final concentrations, that can be used with the formulations of the present invention include, but are not intended to be limited to, ascorbic acid (0.01-0.5%), metal ascorbates (0.2-1.0%), sodium bisulfite (0.05-1.0%), sodium metabisulfite (0.025-0.2%), ascorbyl palmitate (0.01-1.0%), sodium sulfite (0.01-1.0%),

sodium formaldehyde sulfoxylate (0.005-0.15%), acetone sodium bisulfite (0.2%), tocophenol (0.05-0.5%), cysteine (0.1-0.5%), thioglycerol (0.1-0.5%), monothioglycerol (0.1-0.5%), nordhydroguaianetic acid (0.01%), ascorbic acid esters (0.015%), thioglycolic acid (0.01-1.0%), thiortdipropionic acid (0.01-1.0%), and dilaurylthiodipropionate (0.01-1.0%).

The preferred antioxidants are the metal ascorbates such as the alkali or alkaline earth metal ascorbates. Most preferably, the metal ascorbate is sodium ascorbate (Aldrich Chemical Company, Inc., Milwaukee, WI., 53233).

An advantage of the formulations of the present invention is that the formulations may be terminally sterilized. With respect to formulations, terminal sterilization generally includes, but is not intended to be limited to, autoclaving, gamma radiation and electron beam sterilization techniques. For purposes of this disclosure, terminal sterilization will primarily refer to autoclaving processes. Typically, formulations employing higher concentrations of sodium ascorbate, such as 10 mg/ml, employ aseptic fill techniques.

Terminal sterilization provides a much desirable SAL (10^{-6}), than that of aseptic filling (10^{-3}). Terminal sterilization of formulations containing higher sodium ascorbate concentrations may result in discoloration of product due to sodium ascorbate degradation. The American Public Health Association (APHA) publishes guidelines for products including color standards. The APHA color standard for such products are well known to those skilled in the pharmaceutical arts. Color standard information may be found in the 1995 US Pharmacopiea/National Formulary, Edition No. 23, pages 1779-1780.. Generally, in the formulations of the present invention, an APHA of less than 300 is desirable.

Higher concentrations of sodium ascorbate in the formulations of the present invention, such as 10 mg/ml, may be used. However, higher concentrations, such as 10 mg/ml, may require aseptic filling and a less desirable SAL. More preferably, the formulations of the present invention contain

lower sodium ascorbate levels to allow for terminal sterilization of the end product. Concentrations of sodium ascorbate in formulations of the present invention can be controlled to provide an APHA of less than 300.

Stable aqueous formulations of the present invention comprise 1α, 25-dihydroxycholecalciferol, a solubilizing agent, an antioxidant, and are essentially free of a buffer and a chelating agent. Two examples of the preferred formulations of the present invention are presented in Table 1:

Table 1

	Solution comprising 1 micrograms/milliliter (μg/ml) of 1α, 25-dihydroxycholecalciferol	Solution comprising 2 micrograms/milliliter (μg/ml) of 1α, 25-dihydroxycholecalciferol
1α, 25-dihydroxycholecalciferol in Tween® 20 concentrate, 575 μg/g	2.0 milligrams/milliliter (mg/ml)	4.0 milligrams/milliliter (mg/ml)
Tween® Polysorbate 20	2.0 mg/ml	0.0 mg/ml
Sodium Ascorbate	2.5 mg/ml	2.5 mg/ml
Hydrochloric Acid	q.s.	q.s.
Sodium Hydroxide	q.s.	q.s.
Water for Injection	q.s.	q.s.

Another advantage of the formulations of the present invention is the elimination of chelating agents and buffers

which are often used as product stabilizers. Buffers often used in formulations include, but are not limited to, acetate, citrate, glutamate, and phosphate buffers. The formulations of the present invention are essentially free of chelating agents and buffers which result in at least a two fold or greater decrease in aluminum levels in the final product. Preferably, the formulations of the present invention are completely free of chelating agents and buffers.

Generally, aluminum content levels exceed 1000 parts per billion (ppb) in formulations which include buffers and/or chelating agents. As storage time of the formulation increases, the aluminum content in the formulation may increase as well. Aluminum from the glass ampoule may be extracted into the formulation over time. Generally, buffers themselves contain substantial quantities of aluminum so that when they are used in pharmaceutical formulations, they impart aluminum as well.

Preferably, the formulations of the present invention have initial aluminum content levels of less than 1000 ppb. More preferably, the formulations of the present invention have less than 100 ppb of aluminum. Most preferably, the formulations of the present invention have less than 10 ppb of aluminum. The term "initial" in this disclosure with respect to testing parameters (e.g., aluminum levels and pH) refers to testing the end product after sterile filtration or autoclaving.

In the preferred embodiments of this invention, the stable, aqueous, dilute, sterile 1α,25-dihydroxycholecalciferol solution for parenteral administration may be supplied in unit dose 1 ml amber glass ampoules, having the headspace filled by an inert atmosphere such as nitrogen or argon, and which are stored at temperatures of from about 15° to 30° C. in a darkened area.

Each 1 ml of solution preferably contains 1.0 or 2.0 μg of 1α,25-dihydroxycholecalciferol, 4.0 mg of TWEEN®20 (Polysorbate 20, Sigma Chemical Co., St. Louis, Mo., 63178) nonionic surfactant, 2.5 mg of sodium ascorbate, hydrochloric acid q.s., sodium hydroxide q.s., and water for injection q.s. The

pH of the final product is from about 5.8 to about 7.8 and most preferably from about 5.8 to about 7.0. It is understood by those skilled in the art that all components of the present formulations are of a pharmaceutically acceptable grade and quality.

Ampoules containing the formulations of the present invention may be aseptically filled using a series of filters to assure a sterility assurance level (SAL) of 1 X 10 $^{-3}$. More preferably, ampoules containing the formulations of the present invention may be filled and then terminally sterilized to provide a SAL of 1 X 10^{-6}. For example, an aqueous solution of a formulation of the present invention may be filtered, using a 0.45 micrometer (μm) or finer membrane filter (Millipore Corporation, Bedford, MA. 01730), into ampoules. The ampoules may be sealed under a nitrogen blanket and terminally sterilized.

Terminal sterilization of the final product may be done at a Fo of 8 or a Fo of 16. The term "Fo" relates to the degree of sterilization and is well known to those skilled in the art. A Fo of 8 denotes a sterilization cycle run at 121.11° C, with saturated steam for 8 minutes, while a Fo of 16 denotes a cycle at 121.11° C, with saturated steam for 16 minutes.

The stabilized, aqueous solution of the present invention may be prepared under an inert atmosphere, such as nitrogen or argon, in order to insure that oxidation of sodium ascorbate and 1α,25-dihydroxycholecalciferol is minimized during terminal sterilization and end product storage. Preferably, the concentration of oxygen in the headspace is in the range of <3-7%. More preferable is a headspace oxygen concentration of <3-4%. The higher the percentage of oxygen in the headspace may result in a larger pH change upon terminal sterilization and end product storage. In the following Examples, formulations that were stored (e.g., 3, 4, 6, 12, 18, and 24 months), were filled into glass ampoules and stored at the appropriate temperature until tested.

Example 1

An aqueous solution "A" containing 2 μg/ml of 1α,25-dihydroxycholecalciferol, 15.7 mg/ml of citrate buffer, and 2.5 mg/ml of sodium ascorbate was prepared. A second solution "B" was prepared that was identical to solution "A" but also contained the chelating agent, EDTA (1.1 mg/ml). Samples were tested initially and at 3 and 4 months storage at 25° C for aluminum content. Aluminum content was determined by using atomic absorption spectrophotometry. Aluminum content is reported in parts per billion (ppb). The aluminum content of formulations tested throughout this disclosure were measured using a Perkin-Elmer Zeeman/3030 Atomic Absorption Spectrophotometer (Perkin-Elmer, Norwalk, CN., 06856). The results are presented in Table 2. The aluminum content of solution "B" is initially approximately 10% greater than solution "A".

Table 2

Time of Testing	Solution A (ppb)	Solution B (ppb)
Initial	1065	1182
3 months	1460	1920
4 months	1330	1950

Example 2

An aqueous solution "A" containing 2 μg/ml of 1α,25-dihydroxycholecalciferol with 2.5 mg/ml sodium ascorbate was prepared. A second solution "B" was prepared that was identical to solution "A" but also contained the chelating agent, EDTA (1.1

mg/ml). The solutions for storage were aseptically filled into ampoules. The samples were tested for aluminum content initially and at 3, 6, and 12 months storage at 25° C. Aluminum content was determined by using atomic absorption spectrophotometry. Aluminum content is reported in parts per billion (ppb). The results are presented in Table 3. The aluminum content in solution "B" at initial testing and at 3, 6, and 12 and 24 months storage was at least 2 times greater than solution "A".

Table 3

Time of Testing	Aluminum content of Solution A (ppb)	Aluminum content of Solution B (ppb)
Initial	<5	24
3 months	167	335
6 months	221	537
12 months	388	1270
24 months	542	2290

Example 3

Both solution "A" and solution "B" samples from Example 2 were terminally sterilized at a Fo of 8 and 16 to determine the effects of terminal sterilization and storage on pH and aluminum content. Samples were tested for pH and aluminum

initially after terminal sterilization and at 3, 6, and 12 months storage at 25° C.

Solution "A" was terminally sterilized at an Fo of 8. Solution "C" was the same as solution "A" but was terminally sterilized at an Fo of 16. Solution "B" was terminally sterilized at an Fo of 8 and solution "D" was the same as solution "B" but was terminally sterilized at an Fo of 16. The lots containing EDTA (solution "B" and solution "D") showed approximately a 2-5.5 times increase in aluminum content over the non-EDTA containing samples (solution "A" and solution "C"). In addition, a drop in pH in all samples was seen which may be attributed to oxidation of sodium ascorbate. The pH of solution "A" and "C" were consistently lower than solutions "B" and "D" which was probably due to the buffering capability provided by EDTA. Results are shown in Table 4. The value for solution "A" at 18 months is the average of 2 readings.

Table 4

Time of Testing	Solution A		Solution B		Solution C		Solution D	
	Aluminum Content (ppb)	pH	Aluminum Content (ppb)	pH	Aluminum Content (ppb)	pH	Aluminum Content (ppb)	pH
Initial	73	6.7	159	6.7	79	6.5	282	6.7
3 months	174	6.3	359	6.5	218	6.3	646	6.5
6 months	230	6.3	634	6.7	205	6.4	725	6.7
12 months	391	6.4	1375	6.6	424	6.4	1445	6.6
18 months	518	6.4	2325	6.6	675	6.4	2305	6.7

Example 4

Twelve different lots were prepared to determine the pH changes over time with different oxygen (O_2) concentrations in the headspace of the ampoule. The pH of the different lots were measured initially and at 3 and 6 months storage at 30° C

The headspace of the ampoules were nitrogen gassed to provide the different O_2 concentrations. Gassing is done by standard operating practices well known to those skilled in the arts. The instrument used to measure percent oxygen headspace was an Oxygen Headspace Analyzer from Toray, Model No. LF-700 (Toray Engineering Co. Ltd., Japan). Three different O_2 concentrations were tested (6±1%, 3±1%, and <3%) with an in-process pH level of 6.5. The in-process pH level was determined by adjusting the final pH of the formulation with dilute sodium hydroxide and/or hydrochloric acid. The in-process pH was taken prior to terminal sterilization.

The changes in pH are generally attributed to the oxidation of sodium ascorbate during terminal sterilization at a Fo of 16. 1α,25-dihydroxycholecalciferol is sensitive to oxidation degradation. A higher in-process pH and well-controlled headspace gassing may be desirable with formulations of the present invention due to the low buffering capacity of the formulations. The results are presented in Table 5.

Table 5

Time of Testing	<3%	3±1 %	6±1 %
In Process			
Initial	6.2	6.0	5.8
3 months	5.9	6.0	5.8
6 months	6.2	6.1	5.9

Example 5

The solubilizing agent, Tween® 20, was heated in a glass-lined or 316 or higher temper grade stainless steel vessel to a temperature of between 50° to 75° C. Nitrogen gas protection was maintained in the vessel headspace throughout the preparation. After the solubilizing agent was heated to the desired temperature, the 1α,25-dihydroxycholecalciferol was added to the heated solubilizing agent with mixing. Mixing was continued until the 1α,25-dihydroxycholecalciferol dissolved (approximately 10-15 minutes) and the mixture was uniform thereby providing a concentrate containing 575 μg 1α,25-dihydroxycholecalciferol/gram of Tween® 20. The concentrate was then allowed to cool.

Thereafter, an aqueous solution was prepared by heating approximately 110% of the total volume of water for injection in a mixing tank to a temperature of not less than 85° C. The water was cooled to a temperature of between 30° to 45° C while nitrogen bubbling was continued. Before the water reached 30° C, 20% of the final volume in the mixing tank was transferred to a separate covered tank that had been pregassed with nitrogen. The water in the separate covered tank was used to q.s the aqueous solution in the mixing tank.

When the water in the mixing tank had cooled to 20° to 30° C, sodium ascorbate (2.5 mg/ml) was added and mixed until dissolved. Tween® 20 (2 mg/ml) was then added and mixed. The nitrogen bubbling was terminated and sparging of the headspace with nitrogen gas begun. 1α,25-dihydroxycholecalciferol/solubilizing agent concentrate was added (2 mg/ml) and gently mixed until it dissolved into a uniform solution (approximately 10-15 minutes). The 1α,25-dihydroxycholecalciferol/solubilizing agent concentrate was added in sufficient quantity to give a final product 1α,25-dihydroxycholecalciferol concentration of 1 μg/ml.

The aqueous solution in the mixing tank was q.s. to its final volume with water from the separate covered tank. The water was mixed gently into the aqueous solution to provide a uniform solution. The pH was adjusted with the addition of hydrochloric acid and/or sodium hydroxide. The mixing tank was sealed and the contents nitrogen sparged.

The aqueous solution was then transfer filtered with a 0.45 μm membrane into a glass-lined holding tank. The fill lines were flushed with nitrogen gas to prevent oxidation of the product prior to filtering. The aqueous solution in the holding tank was filtered with a 0.45 μm (or finer) membrane filter under an inert atmosphere and then filled in the desired volume into sterile, dry one ml ampoules.

Each ampoule was flushed with nitrogen gas prior to filling. The ampoules were filled and headspace nitrogen gassed prior to sealing the container. The ampoules were then terminally sterilized at a Fo of 8.

Example 6

The 1α,25-dihydroxycholecalciferol/polysorbate 20 concentrate (575 μg 1α,25-dihydroxycholecalciferol/g of polysorbate 20) was prepared as described in Example 5.

Thereafter, an aqueous solution was prepared by heating approximately 110% of the total volume of water for injection in a mixing tank to a temperature of not less than 85° C. The water was cooled to a temperature of between 30° to 45° C while nitrogen bubbling was continued. Before the water reached 30° C, 20% of the final volume in the mixing tank was transferred to a separate covered tank that had been pregassed with nitrogen. The water in the separate covered tank was used to q.s the aqueous solution in the mixing tank.

When the water in the mixing tank had cooled to 20° to 30° C, sodium ascorbate (2.5 mg/ml) was added and mixed until dissolved. The nitrogen bubbling was terminated and sparging of

the headspace with nitrogen gas begun. 1α,25-dihydroxycholecalciferol/solubilizing agent concentrate was added (4 mg/ml) and gently mixed until it dissolved into a uniform solution (approximately 10-15 minutes). The 1α,25-dihydroxycholecalciferol/solubilizing agent concentrate was added in sufficient quantity to give a final product 1α,25-dihydroxycholecalciferol concentration of 2 μg/ml.

The aqueous solution in the mixing tank was q.s. to its final volume with water from the separate covered tank. The water was mixed gently into the aqueous solution to provide a uniform solution. The pH was adjusted with the addition of hydrochloric acid and/or sodium hydroxide. The mixing tank was sealed and the contents nitrogen sparged.

The aqueous solution was then transfer filtered with a 0.45 μm membrane into a glass-lined holding tank. The fill lines were flushed with nitrogen gas to prevent oxidation of the product prior to filtering. The aqueous solution in the holding tank was further filtered with a 0.45 μm (or finer) membrane filter. The headspace in the holding tank was sparged with nitrogen gas.

Each ampoule was flushed with nitrogen gas prior to filling. The ampoules were filled and headspace nitrogen gassed prior to sealing the container. The ampoules were then terminally sterilized at a Fo of 8.

WE CLAIM:

1. A pharmaceutical formulation having a pH of from about 5.8 to about 7.8, comprising a therapeutically effective amount of 1α, 25 dihydroxycholecalciferol, a solubilizing agent, an antioxidant, and essentially free of a buffer and a chelating agent, said pharmaceutical formulation having at least a two fold decrease in aluminum content as compared to said pharmaceutical formulation containing a buffer and a chelating agent.

2. A pharmaceutical formulation in accordance with claim 1 wherein, said solubilizing agent is selected from the group consisting of dimethylacetamide, polyethylene glycol 400 (PEG 400), polyethylene glycol 200 (PEG 200), ethanol, isopropanol, 1,3-butanediol, propylene glycol, dimethylsulfoxide, glycerin, water, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, polyoxalkylene compounds, partial esters of fatty acids, partial esters of polyhydric alcohols, and anhydrides of polyhydric alcohols etherified with polyalkylene oxides.

3. A pharmaceutical formulation in accordance with claim 1 wherein, said antioxidant is selected from the group consisting of ascorbic acid, metal ascorbate, sodium bisulfite, sodium metabisulfite, ascorbyl palmitate, sodium sulfite, sodium formaldehyde sulfoxylate, acetone sodium bisulfite, thioglycerol, thioglycolic acid, thiodipropionic acid, and dilaurylthiodipropionate.

4. A pharmaceutical formulation in accordance with claim 1 wherein, said solubilizing agent is a nonionic surfactant.

5. A pharmaceutical formulation in accordance with claim 4 wherein, said nonionic surfactant is polyoxyethylenesorbitan monolaureate.

6. A pharmaceutical formulation in accordance with claim 3 wherein, said metal ascorbate is selected from the group consisting of alkali and alkaline earth metal ascorbates.

7. A pharmaceutical formulation in accordance with claim 6 wherein, said metal ascorbate is sodium ascorbate.

8. A sterile, aqueous solution having a pH from about 5.8 to about 7.8, comprising a therapeutically effective amount of 1α, 25 dihydroxycholecalciferol, a solubilizing agent and an antioxidant and essentially free of a buffer and a chelating agent, said pharmaceutical formulation having at least a two fold decrease in aluminum content as compared to said pharmaceutical formulation containing a buffer and a chelating agent.

9. A sterile, aqueous solution in accordance with claim 8 wherein, said solubilizing agent is selected from the group consisting of dimethylacetamide, polyethylene glycol 400 (PEG 400), polyethylene glycol 200 (PEG 200), ethanol, isopropanol, 1,3-butanediol, propylene glycol, dimethylsulfoxide, glycerin, water, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, polyoxalkylene compounds, partial esters of fatty acids, partial esters of polyhydric alcohols, and anhydrides of polyhydric alcohols etherified with polyalkylene oxides.

10. A sterile, aqueous solution in accordance with claim 9 wherein, said antioxidant is selected from the group consisting of ascorbic acid, metal ascorbate, sodium bisulfite, sodium metabisulfite, ascorbyl palmitate, sodium sulfite, sodium formaldehyde sulfoxylate, acetone sodium bisulfite, thioglycerol, thioglycolic acid, thiodipropionic acid, and dilaurylthiodipropionate.

11. A sterile, aqueous solution in accordance with claim 9 wherein, said solubilizing agent is a nonionic surfactant.

12. A sterile, aqueous solution in accordance with claim 11 wherein, said nonionic surfactant is polyoxyethylenesorbitan monolaureate.

13. A sterile, aqueous solution in accordance with claim 10 wherein, said metal ascorbate is selected from the group consisting of alkali and alkaline earth metal ascorbates.

14. A sterile, aqueous solution in accordance with claim 13 wherein, said metal ascorbate is sodium ascorbate.

15. A process for preparing a sterile, aqueous solution comprising a therapeutically effective amount of 1α, 25 dihydroxycholecalciferol comprising the steps of:

a). preparing a concentrate of 1α, 25 dihydroxycholecalciferol and a solubilizing agent;

b). adding said concentrate in a predetermined amount to an aqueous solution of an antioxidant, mixing said concentrate and said aqueous solution of said antioxidant to provide a uniform mixture, said uniform mixture prepared under an inert atmosphere, said uniform mixture having a pH of from about 5.8 to about 7.8 by addition of hydrochloric acid or sodium hydroxide, said uniform mixture essentially free of a buffer and a chelating agent and having at least a two fold decrease in aluminum content as compared to said uniform mixture containing a buffer and a chelating agent; and

c). sterile filtering said uniform mixture.

16. A process in accordance with claim 15 wherein, said uniform mixture is terminally sterilized.

17. An aqueous formulation having a pH of from about 5.8 to about 7.8, containing 1 micrograms/milliliter (μg/ml) of 1α, 25-dihydroxycholecalciferol comprising:

2.0 milligrams/milliliter of a 1α, 25-dihydroxycholecalciferol/Tween® 20 concentrate, said concentrate containing 575 micrograms of 1α, 25-dihydroxycholecalciferol per gram of concentrate;

2.0 mg/ml of Tween® 20;

2.5 mg/ml of Sodium Ascorbate; and said aqueous formulation essentially free of a buffer and a chelating agent, said aqueous formulation having at least a two fold decrease in aluminum content as compared to said aqueous formulation containing a buffer and a chelating agent.

18. A process in accordance with claim 15 wherein, said uniform mixture is terminally sterilized.

19. An aqueous formulation having a pH of from about 5.8 to about 7.8, containing 2 micrograms/milliliter (μg/ml) of 1α, 25-dihydroxycholecalciferol comprising:

4.0 milligrams/milliliter of a 1α, 25-dihydroxycholecalciferol in Tween® 20 concentrate, said concentrate containing 575 micrograms of 1α, 25-dihydroxycholecalciferol per gram of concentrate;

2.5 milligrams/milliliter of Sodium Ascorbate and said aqueous formulation essentially free of a buffer and a chelating agent, said aqueous formulation having at least a two fold decrease in aluminum content as compared to said aqueous formulation containing a buffer and a chelating agent.

20. A process in accordance with claim 15 wherein, said uniform mixture is terminally sterilized.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/07074

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/59 A61K9/08 A61K9/00 A61K47/22 A61K47/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9342 Derwent Publications Ltd., London, GB; Class A96, AN 93-331356 XP002010495 & JP,A,05 238 936 (DAINIPPON PHARM CO LTD) , 17 September 1993 see abstract	1-16,18, 20
X	EP,A,0 651 994 (DIETL HANS) 10 May 1995 *cf. page 4, lines 33-48, lines 55-56, page 5, lines 6-7, claims*	1-16,18, 20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 August 1996	22.08.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Stoltner, A

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INTERNATIONAL SEARCH REPORT

Int...ational Application No
PCT/US 96/07074

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	US,A,4 308 264 (CONWAY NANCY M ET AL) 29 December 1981 *cf. abstract, col. 2, summary, col. 3, lines 1-50, col. 4, 2nd para., col. 5, table , claims*	1-15,18, 20

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/07074

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